PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/52, A61K 38/19

A1

(11) International Publication Number: WO 98/14476

(43) International Publication Date: 9 April 1998 (09.04.98)

(21) International Application Number: PCT/US97/16196

(22) International Filing Date: 12 September 1997 (12.09.97)

(30) Priority Data:
08/726,123 4 October 1996 (04.10.96) US

(71) Applicant *(for all designated States except US)*: AMGEN INC. [US/US]; Amgen Center, 1840 De Havilland Drive, Thousand Oaks, CA 91320–1789 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BREMS, David, N. [US/US]; 3778 Calle Clara Vista, Newbury Park, CA 91320 (US). TREUHEIT, Michael, J. [US/US]; 824 Pamela Wood Street, Newbury Park, CA 91320 (US).

(74) Agents: ODRE, Steven, M. et al.; Amgen Inc., Amgen Center, 1840 De Havilland Drive, Thousand Oaks, CA 91320–1789 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PHARMACEUTICAL COMPOSITIONS CONTAINING AN MPL LIGAND

(57) Abstract

The subject invention relates to compositions of mpl ligands, comprising a full–length or truncated mpl ligand having a sequence of amino acids corresponding to amino acids 7–151 through 1–332, inclusive, of native human mpl ligand, optionally covalently linked to at least one water–soluble polymer; a buffering agent selected from glutamate, phosphate, histidine, imidazole, and acetate; an excipient selected from sorbitol, sucrose, mannitol, glycerol, polyethylene glycol, and non–polar amino acids; optionally, a detergent or lipid such as Tween; optionally, an antioxidant or chelating agent selected from glutathione, methionine, citrate and EDTA; and having a pH preferably ranging from 5.0 to 6.0 (inclusive). Such compositions may be liquid (preferably, aqueous), frozen (preferably, aqueous), or lyophilized.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

PHARMACEUTICAL COMPOSITIONS CONTAINING AN MPL LIGAND

Field of the Invention

The present invention relates to compositions containing an mpl ligand, which compositions are suitable for pharmaceutical administration.

Background of the Invention

The native human mpl ligand is a recently cloned cytokine that appears to be the major regulator of circulating platelet levels. See Bartley, T.D. et al., *Cell* **77**:1117-1124 (1994); Lok, S. et al., *Nature* **369**:565-568 (1994); de Sauvage, F.J. et al., *Nature* **369**:533-538 (1994); Miyazake, H. et al., *Exp. Hematol.* **22**:838 (1994); and Kuter, D.J. et al., *PNAS USA*, **91**:11104-11108 (1994). Native human mpl ligand, also referred to as thrombopoietin (TPO) and megapoietin, is a protein having 332 amino acids in total.

Recombinant mpl ligand produced in both Chinese Hamster Ovary (CHO) and *E. coli* cells has been demonstrated to have a biological activity of specifically stimulating or increasing megakaryocytes and/or platelets *in vivo* in mice, rats and monkeys. See e.g., Hunt, P. et al., *Blood* **84**(10):390A (1994). Human mpl ligands that have been truncated from the C-terminus by up to 181 amino acids retain biological activity *in vivo*. The resulting mpl ligands have sequences that correspond to amino acids 1 - 151 up to 1 - 331 of the full-length human sequence. It is also possible to remove up to the first six amino acids at the N-terminus of the human mpl ligand protein and retain biological activity. Therefore, it appears that biological activity is retained within amino acids 7 to 151 (inclusive) of the mature amino acid sequence of human mpl ligand.

Derivatives of mpl ligands have been demonstrated to have advantageous activity to stimulate production of megakaryocytes and/or platelets in *in vivo* tests. See published PCT Application WO 95/26746. In particular, mpl ligands derivatized with water soluble polymers such as polyethylene glycol ("PEG") moieties are of interest in a clinical setting because they are long-lived and active *in vivo*.

Compositions containing mpl ligands and related derivatives have been disclosed in a general sense. See published PCT Applications WO 95/26746, WO 95/21919, WO 95/18858, and WO 95/21920. However, controlled experiments resulting in a determination of which compositions containing mpl ligands are suitably stable for pharmaceutical use, as set forth herein, have not been previously reported. Such compositions are important for practical application of mpl ligands to patients such as humans. Thus, there continues to exist a need for such compositions in the art for use in administering mpl ligands to patients so as to result in an increase in platelets.

Summary of the Invention

Accordingly, it is an object of this invention to provide stable compositions that are pharmaceutically acceptable, which include mpl ligands.

It is another object of this invention to provide compositions that contain mpl ligands for administration to patients.

In one embodiment, the subject invention relates to compositions of mpl ligands, comprising a full-length or truncated mpl ligand having a sequence of amino acids corresponding to amino acids 7-151 through 1-332, inclusive, of native human mpl ligand, optionally covalently linked to

at least one water-soluble polymer; a buffering agent selected from glutamate, phosphate, histidine, imidazole, and acetate; an excipient selected from sorbitol, sucrose, mannitol, glycerol, polyethylene glycol, and non-polar amino acids; optionally, a detergent such as Tween; optionally, an antioxidant or chelating agent selected from glutathione, methionine, citrate and EDTA; and having a pH preferably ranging from 5.0 to 6.0 (inclusive). Such compositions may be liquid (preferably, aqueous), frozen (preferably, aqueous), or lyophilized.

Other aspects of the present invention are set forth in the detailed disclosure provided hereinbelow.

Brief Description of the Drawings

FIG. 1 shows the sequences of the native human cDNA for mpl ligand and the corresponding protein (SEQ ID NOS: 1 and 2). The sequences include a leader sequence (amino acids -21 through -1, inclusive) that is cleaved in vivo from the cDNA encoded protein to yield the mature protein.

Detailed Description of the Invention

The subject invention provides compositions including mpl ligands along with other agents that result in stable, biologically active compositions suitable for administration to subjects such as human beings.

In a first embodiment, the subject invention relates to compositions of an mpl ligand. By "mpl ligand" in its broadest sense is meant any proteinaceous molecule that has the ability to specifically bind to and activate the mpl receptor to result in the stimulation in vivo of megakaryocyte and/or platelet production. In a preferred embodiment, the mpl ligand has an amino acid sequence identical to one obtainable from a human, such as amino acids

1-332 of the native human sequence (SEQ ID NO: 2). In another preferred embodiment, the mpl ligand has an amino acid sequence identical to at least amino acids 7-151 of SEQ ID NO: 2, preferably 1-171 ± 20 amino acids (i.e., amino acids 1-151 through 1-191) particularly preferably 1-161 ± 10 amino acids corresponding to SEQ ID NO: 2. Some specific preferred species of mpl ligands are the following: amino acids 1-151, 1-152, 1-153, 1-154, 1-163, 1-174, 1-191, 1-232, 1-244 of SEQ ID NO: 2. The most preferred species has amino acids 1-163 of SEQ ID NO: 2.

The mpl ligands may also be derivatized with one or more water soluble polymers, such as one or more polyethylene glycol (PEG) groups. The polymer selected should be water soluble so that the mpl ligand to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Examples of water soluble polymers are set forth in published PCT Application WO 95/26746, which is hereby incorporated by reference.

The water soluble polymers may be attached using chemical reactions such as those described in published PCT Application WO 95/26746. Preferred attachment chemistries are acylation and alkylation. The mpl ligand derivatives of this invention may be attached to multiple polymeric molecules, for example, they may contain 2-6, preferably 2-5, polymer groups attached. The polymer groups are usually attached to the protein at the alpha or epsilon amino groups of amino acids, but it is also contemplated that the polymer groups could be attached to any amino group attached to the protein which is sufficiently reactive to become attached to a polymer group under suitable reaction conditions.

In a preferred embodiment, a single polymer molecule is attached to the mpl ligand. In such cases, the polymer selected to react with the mpl ligand should be modified to have a single reactive group, such as an active

ester for acylation or an aldehyde for alkylation, so that the degree of polymerization may be controlled.

The polymer may be branched or unbranched. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable. The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol, monomethoxy-polyethylene glycol, dextran, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol. For derivatization of the mpl ligand via an acylation reaction, the polymer(s) selected should have a single reactive ester group. For derivatization of the mpl ligand via a reductive alkylation reaction, the polymer(s) selected should have a single reactive aldehyde group. Generally, the water soluble polymer will not be selected from naturally-occurring glycosyl residues since these are usually made more conveniently by mammalian recombinant expression systems. The polymer may be of any molecular weight as long as it does not substantially interfere with or abolish biological activity of the resulting mpl ligand derivative.

A particularly preferred water-soluble polymer for use herein is polyethylene glycol, abbreviated PEG. As used herein, polyethylene glycol or PEG is meant to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono-(C1-C10) alkoxy- or aryloxy-polyethylene glycol (see, U.S. Patent 5,252,714).

Pegylation of an mpl ligand may be carried out by any of the pegylation reactions known in the art. See, for example: *Focus on Growth Factors* **3** (2): 4-10 (1992); EP 0 154 316; EP 0 401 384; and the other publications cited herein that relate to pegylation. Preferably, the pegylation

is carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule.

Thus, in a preferred aspect, the present invention relates to pegylated mpl ligand, wherein the PEG group(s) is (are) attached via acyl or alkyl groups. As discussed above, such products may be mono-pegylated or poly-pegylated (e.g., containing 2-6, preferably 2-5, PEG groups). The PEG groups are generally attached to the protein at the alpha or epsilon amino groups of amino acids, but it is also contemplated that the PEG groups could be attached to any amino group attached to the protein, which is sufficiently reactive to become attached to a PEG group under suitable reaction conditions.

Preferably, the PEG group is attached via a reductive alkylation procedure and has a molecular weight of from 5 to 50 kd. In the most preferred embodiment, the PEG-mpl ligand has a PEG group that has an average molecular weight of approximately 20 kd (e.g., 20 kd $\pm$ 2 kd).

A particularly preferred mpl ligand derivative is one corresponding to amino acids 1-163 of SEQ ID NO: 2 attached to a single PEG group on the alpha amino group of the first amino acid, wherein the PEG is attached via a reductive alkylation reaction with a PEG aldehyde reactant. This type of mpl ligand is referred to herein by the abbreviation "PEG-rHuMGDF".

In a preferred embodiment, the mpl ligand is the product of the expression of an exogenous DNA sequence that has been transfected into a host cell; that is, in a preferred embodiment the mpl ligand is a "recombinant mpl ligand". Recombinant mpl ligand may be made in any cells known for this purpose, for example, CHO cells. The preferred host is bacterial, particularly preferably *E. coli* cells. Recombinant mpl ligand is advantageously produced according to the procedures described in the publications cited herein regarding cloning and expression of mpl ligand.

TABLE 1

Buffer	Preferred Conc. Range (mM)	Working Conc. Range (mM)	Exemplary Conc. (mM)
Acetate	5-20	8-12	10
Phosphate	5-20	8-12	10
Histidine	5-20	8-12	10

The pH of the compositions will vary depending on the particular buffer and other factors. The preferred pH range for enhanced stability with appropriate acidic buffers (e.g., acetate) is 4.0-6.0. A more preferred range is 4.5-5.5, with about 5.0 being a most preferred embodiment.

The compositions should also contain an excipient. Some exemplary excipients and representative concentrations are listed in Table 2:

TABLE 2

Excipient	Preferred Conc. Range (W/V)	Working Conc. Range (W/V)	Exemplary Conc. (W/V)
sorbitol	3 - 10%	4 - 6%	5%
sucrose	5 - 10%	8 - 10%	9%
mannitol	3 - 10%	4 - 6%	5%

The excipients will generally be added in an amount so as to result in an isotonic solution.

The compositions may further contain an amino acid, which in some cases will enhance stability. Amino acids may be polar or non-polar, with non-polar amino acids being

preferred. Exemplary polar amino acids are arginine and lysine, and exemplary non-polar amino acids are glycine, proline, and alanine.

An antioxidant or chelating agent may also be included in the compositions of this invention. Preferred antioxidants are: EDTA, ascorbic acid, glutathione, methionine and citrate. Combinations of these agents are also contemplated, for example, citrate plus EDTA. Such agents are included in an amount suitable to reduce or eliminate oxidation of the mpl ligand. Exemplary concentrations are: 0.1 - 10 mM, preferably, 0.5 - 5 mM, typically 1 - 3 mM.

A detergent or lipid may also be included in the compositions of this invention. Some representative detergents are: Tween brand of polysorbate (e.g., Tween 20 and Tween 80); Brij 35; Pluronics (e.g., F-127 and F-68); sodium dodecyl sulfate; Triton (e.g., X-100); dimyristoyl phosphatidyl glycerol (DMPG); PEG castor oil (e.g., PEG-40); oleth-3-phosphate; diethanolamine oleth-10-phosphate; and a mixture (e.g., 1:1) of short, long chain unilamellar vesicles (SLUV) containing, e.g., C8 (caprylic) and C14 (myristic) lipids. These detergents/lipids are generally included in an amount sufficient to prevent loss of mpl ligand due to sticking to surfaces or aggregation. Some exemplary detergent concentrations are 0.004 mg/ml - 50 mg/ml; preferably, 0.004 mg/ml - 10 mg/ml; most preferably, 0.006 - 0.060 mg/ml. The need to include these detergents/lipids will be greater when the concentration of mpl ligand is lower, such as especially $\leq$ 0.2 mg/ml of mpl ligand.

Such compositions may be liquid (preferably, aqueous), frozen (preferably, aqueous), or lyophilized.

With regards to lyophilized compositions, there is the possibility of increased protein aggregation as compared to liquid compositions. A particularly preferred lyophilized composition contains a combination of glutamate, sucrose and

mannitol at a pH within the range of 4.0-6.0. A list of particularly preferred compositions is provided in the following Table 3:

TABLE 3

Material	Range	Example
Glutamate	5-20 mM	10 mM
Sucrose	2-10% (w/v)	6% (w/v)
Mannitol	1-5% (w/v)	2% (w/v)
pH	4.0-6.0	5.0

The compositions of this invention are "stable", by which is meant that they retain at least about 87 %, preferably about 90 %, most preferably about 93 %, of intact mpl ligand derivative after storage for 12 weeks at a temperature of 37°C as analyzed by SEC chromatography only (see Table 4). This degree of stability is important in a practical sense because less stability would result in unacceptable safety concerns for patients.

A "therapeutically effective amount" as used herein refers to that amount which provides a suitable biological effect in a subject, usually a therapeutic effect for a given condition and administration regimen in a patient.

The present compositions can be systemically administered parenterally, intravenously or subcutaneously. When systemically administered, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, physiologically acceptable aqueous solution. The specific route chosen will depend upon the condition being treated. The required dosage will be in amounts sufficient to raise the platelet and/or megakaryocyte levels of patients and will vary depending upon the severity of the condition being treated, the method of administration used and the like.

The conditions to be treated by the methods and compositions of the present invention are generally those which involve an existing megakaryocyte/platelet deficiency or an expected megakaryocyte/platelet deficiency in the future (e.g., because of planned surgery). Such conditions will usually be the result of a deficiency (temporary or permanent) of active mpl ligand *in vivo*. The generic term for platelet deficiency is thrombocytopenia, and hence the methods and compositions of the present invention are generally useful for treating thrombocytopenia.

Thrombocytopenia (platelet deficiencies) may be present for various reasons, including chemotherapy and other therapy with a variety of drugs, radiation therapy, surgery, accidental blood loss, and other specific disease conditions. Exemplary specific disease conditions that involve thrombocytopenia and may be treated in accordance with this invention are: aplastic anemia, idiopathic thrombocytopenia, metastatic tumors which result in thrombocytopenia, systemic lupus erythematosus, splenomegaly, Fanconi's syndrome, vitamin B12 deficiency, folic acid deficiency, May-Hegglin anomaly, Wiskott-Aldrich syndrome, and paroxysmal nocturnal hemoglobinuria. Also, certain treatments for AIDS result in thrombocytopenia (e.g., AZT). Certain wound healing disorders might also benefit from an increase in platelet numbers.

With regard to anticipated platelet deficiencies, e.g., due to future surgery, an mpl ligand analog of the present invention could be administered several days to several hours prior to the need for platelets. With regard to acute situations, e.g., accidental and massive blood loss, an mpl ligand analog could be administered along with blood or purified platelets.

Mpl ligand compositions may also be administered to normal human subjects who plan to donate platelets or other related cells in the future. Administration of a composition

of this invention would increase the amount of platelets and/or related cells that the patient could donate at one time.

The dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician, considering various factors which modify the action of drugs, e.g. the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. Generally, the daily regimen should be in the range of 0.01-1000 micrograms of mpl ligand analog per kilogram of body weight.

The compositions of the present invention may also be employed, alone or in combination with other cytokines, soluble Mpl (i.e., mpl ligand) receptor, hematopoietic factors, interleukins, growth factors or antibodies in the treatment of disease states characterized by other symptoms as well as platelet deficiencies. It is anticipated that such compositions will prove useful in treating some forms of thrombocytopenia in combination with general stimulators of hematopoiesis, such as IL-3 or GM-CSF. Other megakaryocytic stimulatory factors, i.e., meg-CSF, stem cell factor (SCF), leukemia inhibitory factor (LIF), oncostatin M (OSM), or other molecules with megakaryocyte stimulating activity may also be employed with mpl ligand. Additional exemplary cytokines or hematopoietic factors for such co-administration include IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-11, colony stimulating factor-1 (CSF-1), GM-CSF, granulocyte colony stimulating factor (G-CSF), EPO, interferon-alpha (IFN-alpha), IFN-beta, or IFN-gamma. It may further be useful to administer, either simultaneously or sequentially, an effective amount of a soluble mammalian Mpl receptor, which appears to have an effect of causing megakaryocytes to fragment into platelets once the megakaryocytes have reached mature form. Thus,

administration of PEG-mpl ligand (to enhance the number of mature megakaryocytes) followed by administration of the soluble Mpl receptor (to inactivate the analog and allow the mature megakaryocytes to produce platelets) is expected to be a particularly effective means of stimulating platelet production. The dosage recited above would be adjusted to compensate for such additional components in the therapeutic composition. Progress of the treated patient can be monitored by conventional methods.

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

EXAMPLE 1

The following Tables 4 and 5 are a summary of data provided in some of the following examples. In each of these examples, the mpl ligand tested was PEG-rHuMGDF, which contains amino acids 1-163 of SEQ ID NO: 2, mono-pegylated at the alpha amino group of the N-terminal amino acid with a polyethylene glycol group having an average molecular weight of about 20 kDa.

TABLE 4

Formulation	Percent Main Peak as Measured by SEC[1] after 12 weeks at 37°C
Histidine, pH 8.0, 5% Sorbitol	70
Tris, pH 8.0, 5% Sorbitol	47
Phosphate, pH 7.0, 5% Sorbitol	71
Histidine, pH 7.0, 5% Sorbitol	84
Phosphate, pH 6.0, 5% Sorbitol	89
Glutamate/Histidine, pH 6.0, 5% Sorbitol	92
Histidine, pH 6.0, 5% Sorbitol	92
Imidazole, pH 6.0, 5% Sorbitol	92
Glutamate, pH 5.5, 5% Sorbitol	91
Glutamate/Histidine, pH 5.5, 5% Sorbitol	93
Acetate, pH 5.0, 5% Sorbitol	92
Glutamate/Histidine, pH 5.0, 5% Sorbitol	93
Glutamate, pH 5.0, 5% Sorbitol	91
Histidine, pH 5.0, 5% Sorbitol	89
Succinate, pH 5.0, 5% Sorbitol	76
Glutamate, pH 4.0, 5% Sorbitol	87
Succinate, pH 4.0, 5% Sorbitol	81
Acetate, pH 4.0, 5% Sorbitol	77
Tartrate, pH 4.0, 5% Sorbitol	18
Succinate, pH 3.5, 5% Sorbitol	74

[1]Additional stability indicating assays, specifically, reversed phase and cation exchange chromatography, gave similar results to SEC.

The percent decrease in main peak indicates a preferred pH range of 4.0 - 6.0, preferably 5.0 - 6.0. In addition, buffer effects within the pH range 4.0 - 6.0

indicate that certain buffers within this range are not preferred.

TABLE 5

Formulation	Percent Main Peak as Measured by SEC[1] after 12 weeks at 37°C
Acetate, pH 5.0, 5% isotonic polyols (Sorbitol)	92
Acetate, pH 5.0, isotonic saline, both mono- and divalent (NaCl)	10
Acetate, pH 5.0, isotonic polar amino acids (Lysine)	10
Acetate, pH 5.0, isotonic nonpolar amino acids (Glycine)	84

All polyols tested, which include: sorbitol, sucrose, glycerol, mannitol and polyethylene glycol, showed similar results.

All salts (both monovalent and divalent), which include: NaCl, $CaCl_2$, $CuCl_2$, $MgCl_2$, $MnCl_2$, $NiCl_2$, $ZnCl_2$ and $FeCl_2$, showed similar results.

All polar amino acids tested, which include: arginine, and lysine, showed similar results.

All nonpolar amino acids tested, which include: glycine, proline, and alanine, showed similar results.

All antioxidants tested which include: EDTA, ascorbic acid, glutathione, methionine, methionine + EDTA and citrate, did not show substantial increases in the stability of PEG-rHuMGDF beyond A50S (see Example 2 for definition).

EXAMPLE 2

pH Evaluation

A. Starting Material: PEG-rHuMGDF

B. Formulations:

10mM Acetate pH 5.0, 5% Sorbitol	(A50S)
10mM Acetate pH 4.0, 5% Sorbitol	(A40S)
10mM Succinate pH 3.5, 5% Sorbitol	(S35S)
10mM Succinate pH 4.0, 5% Sorbitol	(S40S)
10mM Succinate pH 5.0 5% Sorbitol	(S50S)
10mM Histidine pH 6.0, 5% Sorbitol	(H60S)
10mM Imidazole pH 6.0, 5% Sorbitol	(I60S)
10mM Tartrate pH 4.0, 5% Sorbitol	(T40S)
10mM Glutamate pH 4.0, 5% Sorbitol	(E40S)
10mM Phosphate pH 6.0, 5% Sorbitol	(P60S)
10mM Phosphate pH 7.0, 5% Sorbitol	(P70S)
10mM Tris pH 8.0 5% Sorbitol	(T80S)
10mM Histidine pH 5.0, 5% Sorbitol	(H50S)
10mM Histidine pH 6.0, 5% Sorbitol	(H60S)
10mM Histidine pH 7.0, 5% Sorbitol	(H70S)
10mM Histidine pH 8.0, 5% Sorbitol	(H80S)

C. Vials: 1mL in 3cc vials filled at a protein concentration of 0.5 mg/mL

D. Temp & Time points: 37°C; time points indicated on Tables

E. Analyses: HPLC: Size exclusion chromatography (SEC), reverse phase chromatography (RP), ion exchange chromatography (IEX)

F. Data

Tables 6-11 show the percent main peak by size exclusion, reversed phase and cation exchange chromatography after incubation at 37°C for the times indicated.

pH Evaluation - Percent Main Peak by Size Exclusion Chromatography After Incubation at 37°C for the Time Indicated

TABLE 6

Form.*	Incubation Time					
	T=0	T=2 Weeks	T=4 weeks	T=8 weeks	T=12 Weeks	T=17 Weeks
A40S	94.39	90.86	88.20	82.91	77.21	70.19
S35S	94.90	93.65	90.29	80.95	74.28	62.43
S40S	94.63	92.59	90.20	86.16	80.51	72.34
S50S	94.27	87.53	85.29	80.77	76.45	71.12
H60S	94.99	94.31	93.77	91.74	89.13	85.96
I60S	94.93	94.56	94.20	92.77	91.49	94.83
T40S	89.71	43.46	34.44	24.48	18.10	-
E40S	94.90	94.29	93.65	91.30	86.84	77.94

*Form. = Formulation

TABLE 7

Form.*	Incubation Time							
	T=0	T=3 Days	T=7 Days	T=10 Days	T=2 Weeks	T=3 Weeks	T=6 Weeks	T=12 Weeks
T80S	97.70	93.56	91.28	88.12	86.63	80.25	66.18	46.58
H50S	97.53	94.20	94.22	94.37	94.54	92.94	91.76	89.18
H60S	97.62	97.02	97.24	97.09	94.50	96.19	95.12	91.68
H70S	97.62	96.64	96.75	95.86	95.37	94.33	90.95	84.34
H80S	97.70	-	94.68	93.27	92.03	89.36	81.52	69.65
P60S	97.65	95.55	96.84	96.35	96.38	95.77	93.76	89.16
P70S	97.81	96.45	95.84	94.83	94.12	91.77	84.81	71.14
A50S	97.72	96.28	95.43	95.45	95.63	94.71	93.97	91.65

pH Evaluation - Percent Main Peak by Reversed Phase Chromatography After Incubation at 37C for the Time Indicated

TABLE 8

Form.*	Incubation Time					
	T=0	T=2 Weeks	T=4 weeks	T=8 weeks	T=12 Weeks	T=17 Weeks
A40S	93.49	91.69	84.34	78.87	70.55	64.23
S35S	93.78	90.10	80.86	70.36	59.35	50.98
S40S	93.91	91.61	83.89	77.16	-	59.22
S50S	93.49	92.59	88.93	85.96	79.35	73.71
H60S	93.65	90.54	86.69	86.49	80.88	76.11
I60S	93.68	92.61	87.64	87.34	84.11	79.49
T40S	93.65	88.04	74.51	66.08	53.87	-
E40S	93.67	92.39	86.03	80.64	71.50	65.23

TABLE 9

Form.*	Incubation Time						
	Time Zero	T=3 Days	T=7 Days	T=10 Days	T=2 Weeks	T=6 Weeks	T=12 Weeks
T80S	95.96	93.75	92.68	88.57	87.98	68.58	48.84
H50S	96.14	95.38	95.52	94.70	97.08	92.18	88.45
H60S	95.88	94.32	94.77	94.65	94.08	90.20	84.80
H70S	95.78	92.66	93.84	90.72	90.95	-	70.83
H80S	95.88	94.11	93.23	90.94	89.66	77.82	67.64
P60S	96.49	94.62	94.82	94.18	93.44	86.25	81.09
P70S	96.04	94.98	94.14	92.41	94.37	82.67	71.11
A50S	96.02	95.13	95.57	94.68	96.35	92.78	89.30

pH Evaluation - Percent Main Peak by Cation Exchange Chromatography After Incubation at 37C for the Time Indicated

TABLE 10

Form.*	Incubation Time					
	T=0	T=2 Weeks	T=4 weeks	T=8 weeks	T=12 Weeks	T=17 Weeks
A40S	81.17	73.84	69.66	53.96	43.09	41.14
S35S	82.64	72.97	67.10	52.36	45.84	37.70
S40S	82.16	74.54	70.95	53.81	44.63	34.96
S50S	82.86	75.30	67.91	58.14	49.48	41.49
H60S	82.32	79.60	75.57	70.77	65.47	-
I60S	83.72	80.20	77.95	72.15	64.05	66.01
T40S	79.23	30.25	27.58	19.37	11.92	-
E40S	82.37	75.53	69.71	59.80	50.91	47.72

TABLE 11

Form.*	Incubation Time							
	Time Zero	T=3 Days	T=7 Days	T=10 Days	T=2 Weeks	T=3 Weeks	T=6 Weeks	T=12 Weeks
T80S	87.44	80.38	78.04	76.41	72.76	71.28	54.49	-
H50S	84.42	83.70	83.86	83.23	82.16	78.52	76.07	70.85
H60S	87.86	86.81	85.40	84.56	82.00	78.90	80.64	73.71
H70S	82.99	83.33	81.35	81.01	79.55	72.70	67.95	56.91
H80S	84.08	84.72	81.49	82.17	77.28	64.21	68.21	54.79
P60S	85.68	84.58	83.73	83.22	79.15	75.87	73.38	60.93
P70S	87.60	78.70	82.50	84.04	78.99	77.85	78.90	69.18
A50S	83.00	84.54	81.65	83.81	81.54	77.68	79.50	72.22

EXAMPLE 3

Mpl ligand Concentration Evaluation

A. Starting Material: PEG-rHuMGDF

B. Formulations:

10mM Acetate pH 5.0, 5% Sorbitol, 2.0 mg/mL (20A5S)
10mM Acetate pH 5.0, 5% Sorbitol, 1.0 mg/mL (10A5S)
10mM Acetate pH 5.0, 5% Sorbitol, 0.5 mg/mL (05A5S)
10mM Acetate pH 5.0, 5% Sorbitol, 0.2 mg/mL (02A5S)

C. Vials: 1mL in 3cc vials filled at the protein concentrations indicated

D. Temp & Time points: 37°C; time points indicated on Tables

E. Analyses: HPLC: SEC, RP, IEX

F. Data

Tables 12-14 show the percent main peak by size exclusion, reversed phase and cation exchange chromatography after incubation at 37°C for the times indicated.

Mpl Ligand Concentration Evaluation - Percent Main Peak After Incubation at 37°C for the Time Indicated

TABLE 12

Size Exclusion Chromatography

Form.*	T=0	T=1 Week	T=2 Weeks	T=3 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
02A5S	94.44	94.86	93.84	94.53	94.51	93.90	92.74
05A5S	94.11	93.78	92.86	93.25	93.40	91.55	90.11
10A5S	93.96	92.60	91.69	91.74	91.32	89.20	86.89
20A5S	93.81	91.50	90.25	90.20	89.55	86.89	83.59

*Form. = Formulation

TABLE 13

Reversed Phase Chromatography

Form.*	Incubation Time						
	T=0	T=1 Week	T=2 Weeks	T=3 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
02A5S	94.53	93.41	93.28	92.16	91.05	88.89	87.90
05A5S	94.73	94.44	93.30	92.41	91.97	89.18	85.97
10A5S	94.86	94.56	93.25	92.39	91.98	88.06	87.62
20A5S	94.67	94.12	93.07	92.47	91.79	88.37	87.07

TABLE 14

Cation Exchange Chromatography

Form.*	Incubation Time					
	T=0	T=1 Week	T=2 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
02A5S	79.40	65.75	78.56	79.80	74.42	59.74
05A5S	78.22	67.44	80.26	81.10	76.27	61.39
10A5S	-	66.18	80.80	82.02	75.81	60.72
20A5S	79.52	76.71	82.09	81.38	74.26	60.89

EXAMPLE 4

Excipient Evaluation

A. Starting Material: PEG-rHuMGDF

B. Formulations:

10mM Acetate pH 5.0, 5% Sorbitol, 5 mM EDTA	(A5SE)
10mM Acetate pH 5.0, 2% Alanine	(A5A)
10mM Acetate pH 5.0, 1.6% Glycine	(A5G)
10mM Acetate pH 5.0 2.7% Proline	(A5P)
10mM Acetate pH 5.0, 3.5% Lysine	(A5K)
10mM Acetate pH 5.0, 4.3% Arginine	(A5R)
10mM Glutamate pH 5.0, 9.3% Sucrose	(E5Su)
10mM Glutamate pH 5.0, 5% Sorbitol	(E5S)

C. Vials: 1mL in 3cc vials filled at a protein concentration of 0.5 mg/ml

D. Temp & Time points: 37°C; time points indicated on Tables

E. Analyses: HPLC: SEC, RP, IEX

F. Data

Tables 15-17 show the percent main peak by size exclusion, reversed phase and cation exchange chromatography after incubation at 37°C for the times indicated.

Excipient Evaluation - Percent Main Peak After Incubation at 37C for the Time Indicated.

TABLE 15

Size Exclusion Chromatography

Formulation	Incubation Time				
	T=0	T=2 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
A5R	84.71	21.83	19.01	12.30	-
E5Su	94.30	91.40	91.95	88.36	86.52
A5G	94.57	91.22	91.43	92.26	84.50
A5K	86.75	17.24	15.02	9.76	-
A5SE	93.08	60.01	54.53	43.22	37.75
A5P	94.66	91.97	92.16	87.63	85.05
E5S	94.78	93.07	93.55	90.68	92.28
A5A	94.55	91.57	92.18	88.13	85.89

TABLE 16

Cation Exchange Chromatography

Formulation	Incubation Time				
	T=0	T=2 Weeks	T=4 Weeks	T=8 Weeks	T=12 Weeks
A5R	82.26	28.41	21.32	11.71	-
E5Su	84.93	86.22	88.62	72.16	65.04
A5G	86.17	81.90	66.81	18.95	26.45
A5K	80.88	20.18	14.23	8.75	-
A5SE	84.56	62.75	56.40	36.31	30.38
A5P	86.73	87.58	86.22	69.34	65.76
E5S	87.49	90.23	88.83	75.55	70.01
A5A	86.28	81.31	86.10	70.29	63.69

TABLE 17

Reversed Phase Chromatography

Formulation	Incubation Time			
	T=0	T=2 Weeks	T=4 Weeks	T=12 Weeks
A5R	95.96	92.35	85.68	-
E5Su	96.10	94.57	91.59	77.92
A5G	95.71	69.61	39.86	-
A5K	95.81	88.93	81.30	-
A5SE	95.98	93.60	90.01	-
A5P	95.67	92.98	89.96	78.85
E5S	95.64	93.98	90.61	83.90
A5A	95.78	90.38	86.52	70.00

EXAMPLE 5

Isotonicity Evaluation

A. Starting Material: PEG-rHuMGDF

B. Formulations:

10mM Acetate pH 5.0, 9.3% Sucrose	(A5SU)
10mM Acetate pH 5.0, 5% Mannitol	(A5MA)
10mM Acetate pH 5.0, 140 mM NaCl	(A5N)
10mM Acetate pH 5.0, 2% PEG 8000	(A5P8)
10mM Acetate pH 5.0, 2.5% Glycerol	(A5G)
10mM Acetate pH 5.0, 5% Sorbitol, .01% Tween 20	(A5ST)
10mM Histidine pH 6.0, 5% Sorbitol	(H6S)
10mM Histidine pH 6.0, 5% Sorbitol, .001% Ascorbic Acid	(H6AA)

C. Vials: 1mL in 3cc vials filled at a protein concentration of 0.5 mg/ml

D. Temp & Time points: 37°C; time points indicated on Tables

E. Analyses: HPLC: SEC, RP, IEX

F. Data

Tables 18-20 show the percent main peak by size exclusion, reversed phase and cation exchange chromatography after incubation at 37°C for the times indicated.

Isotonicity Evaluation - Percent Main Peak After Incubation at 37°C for the Time Indicated.

TABLE 18

Size Exclusion Chromatography

Form.*	Incubation Time						
	T=0	T=3.5 Days	T=1 Week	T=2 Weeks	T=3 Weeks	T=7 Weeks	T=12 Weeks
H6S	96.70	96.23	96.30	95.95	95.77	93.37	91.03
H6Aa	96.75	95.66	96.03	95.72	95.29	93.32	90.61
A5N	83.54	30.42	28.04	24.64	20.19	17.62	-
A5Ma	96.55	95.14	95.22	95.02	95.13	93.08	91.42
A5Su	96.36	95.18	95.37	95.16	95.60	93.49	91.89
A5G	96.46	95.29	95.49	95.20	95.59	93.23	91.68
A5P8	94.88	92.89	92.89	92.61	91.62	92.78	82.05
A5ST	96.25	94.83	94.50	94.58	94.37	91.16	87.68

*Form.= Formulation

TABLE 19

Reversed Phase Chromatography

Form.*	Incubation Time					
	T=0	T=3.5 Days	T=1 Week	T=2 Weeks	T=7 Weeks	T=12 Weeks
H6S	95.49	94.39	94.69	85.55	87.60	85.62
H6Aa	95.64	92.00	90.67	93.02	74.22	66.22
A5N	94.94	93.99	92.19	89.52	68.67	-
A5Ma	95.90	95.05	95.29	94.34	90.13	87.22
A5Su	95.55	95.39	94.78	93.47	88.19	82.88
A5G	95.57	94.78	95.51	94.88	90.04	88.64
A5P8	94.75	91.31	87.07	76.93	13.41	-
A5ST	94.57	93.24	93.90	82.75	87.61	85.45

TABLE 20

Cation Exchange Chromatography

Form.*	Incubation Time						
	T=0	T=3.5 Days	T=1 Week	T=2 Weeks	T=3 Weeks	T=7 Weeks	T=12 Weeks
H6S	87.13	85.79	84.67	82.66	82.77	79.32	59.35
H6Aa	85.12	80.75	79.31	75.99	75.23	64.62	45.93
A5N	79.98	39.01	29.14	25.03	20.77	14.67	-
A5Ma	84.73	84.37	84.43	81.92	83.07	78.17	58.52
A5Su	82.98	84.83	83.46	80.76	81.09	76.61	56.85
A5G	82.79	84.69	85.09	81.73	81.61	76.98	59.03
A5P8	85.65	82.26	81.74	77.41	74.77	53.74	4.80
A5ST	85.02	83.83	83.95	80.02	79.79	74.56	55.85

EXAMPLE 6

Anti-oxidant/Chelating Agent Evaluation

A. Starting Material: PEG-rHuMGDF

B. Formulations:

10 mM Acetate pH 5.0, 5% Sorbitol 3 mM glutathione	(A5S GT)
10 mM Acetate pH 5.0, 5% Sorbitol 5 mM methionine	(A5S M)
10 mM Acetate pH 5.0, 5% Sorbitol 5 mM methionine, 1 mM EDTA	(A5S ME)
10 mM Acetate pH 5.0, 5% Sorbitol 1 mM citrate	(A5S C)
10 mM Acetate pH 5.0, 5% Sorbitol 0.5 mM citrate	(A5S 05C)
10 mM Acetate pH 5.0, 5% Sorbitol 1 mM EDTA	(A5S 1E)
10 mM Acetate pH 5.0, 5% Sorbitol 0.5 mM EDTA	(A5S E)

C. Vials: 1mL in 3cc vials filled at a protein concentration of 0.5 mg/ml

D. Temp & Time points: 37°C; time points indicated on Tables

E. Analyses: HPLC: SEC, RP, IEX

F. Data

Tables 21-23 show the percent main peak by size exclusion, reversed phase and cation exchange chromatography after incubation at 37°C for the times indicated.

Anti-Oxidant/Chelating Agent Evaluation - Percent Main Peak After Incubation at 37°C for the Time Indicated.

TABLE 21

Size Exclusion Chromatography

Formulation	Incubation Time			
	T=0	T=2 Weeks	T=4 Weeks	T=9 Weeks
A5SGT	16.69	12.32	8.03	5.91
A5SM	94.53	91.03	89.72	89.00
A5SME	94.88	92.15	90.73	85.74
A5SC	94.30	90.83	88.14	79.61
A5S05C	93.96	90.95	88.98	-
A5S1E	94.88	90.75	89.15	80.63
A5SE	94.80	91.72	89.89	82.06

TABLE 22

Reversed Phase Chromatography

Formulation	Incubation Time				
	T=0	T=2 Weeks	T=4 Weeks	T=9 Weeks	T=12 Weeks
A5SGT	5.69	2.37	2.80	2.60	2.50
A5SM	94.70	91.59	88.67	88.55	86.83
A5SME	94.54	87.66	91.36	81.97	80.39
A5SC	93.98	81.13	90.33	68.01	62.91
A5S05C	94.43	87.01	84.30	79.83	76.35
A5S1E	94.36	86.84	76.43	89.93	84.56
A5SE	94.75	90.27	85.64	81.20	78.96

TABLE 23

Cation Exchange Chromatography

Formulation	Incubation Time			
	T=0	T=2 Weeks	T=4 Weeks	T=9 Weeks
A5SGT	4.66	5.15	0.0	-
A5SM	84.69	81.18	80.96	72.95
A5SME	84.93	78.36	78.26	69.48
A5SC	85.23	72.11	66.46	54.68
A5S05C	86.06	78.00	72.53	66.06
A5S1E	85.38	77.00	76.42	66.21
A5SE	86.51	80.07	77.93	69.79

EXAMPLE 7

Detergent Evaluation

A. Starting Material: PEG-rHuMGDF

B. Formulations:

All forms contain 10mM acetate at pH 5.0, with 5% sorbitol and 0.050 mg/ml PEG-rHuMGDF.

004T20 : 0.004 mg/ml Tween-20
006T20 : 0.006 mg/ml Tween-20
010T20 : 0.010 mg/ml Tween-20
040T20 : 0.040 mg/ml Tween-20
060T20 : 0.060 mg/ml Tween-20
004T80 : 0.004 mg/ml Tween-80
006T80 : 0.006 mg/ml Tween-80
010T80 : 0.010 mg/ml Tween-80
040T80 : 0.040 mg/ml Tween-80
060T80 : 0.060 mg/ml Tween-80

C. Data:

Table 24 shows the results of reverse phase HPLC purity based on the percent main peak:

TABLE 24

FORMULATION	INCUBATION TIME AT 37 DEGREES CELSIUS				
	T=0 WEEKS	T=2 WEEKS	T=4 WEEKS	T=6 WEEKS	T=12 WEEKS
A5S	94.66	94.05	90.67	90.90	89.07
004T20	94.68	94.79	91.25	91.02	88.49
006T20	94.69	93.99	91.48	90.46	88.43
010T20	94.89	94.30	91.33	90.76	88.47
040T20	94.59	92.95	90.63	89.88	87.58
060T20	94.61	92.62	90.57	89.49	86.72
004T80	94.44	94.35	90.91	90.80	88.48
006T80	94.43	94.05	91.01	90.18	88.08
010T80	94.89	94.22	90.69	90.22	88.04
040T80	94.45	92.64	89.74	89.26	86.56
060T80	94.19	88.38	88.67	87.64	84.53

D. Results:

Detergents such as Tween may be included in PEG-rHuMGDF formulations to enhance physical stability and recovery without detrimental effects to chemical stability. Tween-20 and Tween-80 may be added to PEG-rHuMGDF formulations up to final concentrations of about 0.060 mg/ml without causing excessive methionine oxidation. Tween-20 and Tween-80 are most effective in the concentration range of 0.006 mg/ml to 0.060 mg/ml.

EXAMPLE 8

Lyophilized Compositions

The following Table 25 is a summary of data obtained for lyophilized compositions including an mpl ligand. In each of these examples, the mpl ligand tested was PEG-rHuMGDF, which contains amino acids 1-163 of SEQ ID NO: 2, mono-PEGylated at the alpha amino group of the N-terminal amino acid with a polyethylene glycol group having an average molecular weight of about 20 kDa. For Tables 25-27, the lyophilized PEG-rHuMGDF was reconstituted with about 1 ml of water for injection prior to analysis and the percent main peak represents the recovery of PEG-rHuMGDF as a consequence of lyophilization.

TABLE 25

Formulation	% main peak by SEC
10mM histidine, 5% mannitol	88
10mM histidine, 4% mannitol, 1% sucrose	93

Note: Mpl ligand concentration was 0.5 mg/ml.

For the lyophilized samples no additional physical stability was achieved in pH's 6, 7 and 8 as measured by size exclusion.

Range of sucrose concentrations studied:

TABLE 26

Sucrose Conc.	% main peak by SEC
2%	91
4%	94
5%	97
6%	95

Range of buffers (10mM concentration) studied:

TABLE 27

Formulation	% main peak by SEC HPLC
histidine, 3.8% mannitol, 2% sucrose, pH 5	87
citrate, 3.8% mannitol, 2% sucrose, pH 5	87
acetate, 3.8% mannitol, 2% sucrose, pH 5	79
succinate, 3.8% mannitol, 2% sucrose, pH 5	88
MES, 3.8% mannitol, 2% sucrose, pH 5	88
phosphate, 3.8% mannitol, 2% sucrose, pH 7	87
phosphate, 3.8% mannitol, 0.5% glycine, pH 7	80

All stabilizing agents such as amino acids (e.g. isotonic arginine, lysine, proline and histidine) and

amorphous agents (e.g., trehalose and PEG) did not show improved stability during lyophilization.

Formulation with best main peak recovery and lowest levels of aggregation:

10mM glutamate, 6% sucrose, 2% mannitol, pH 5.0.

While the invention has been described in what is considered to be its preferred embodiments, it is not to be limited to the disclosed embodiments, but on the contrary, is intended to cover various modifications and equivalents included within the spirit and scope of the appended claims, which scope is to be accorded the broadest interpretation so as to encompass all such modifications and equivalents.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: AMGEN INC.

(ii) TITLE OF INVENTION: PHARMACEUTICAL COMPOSITIONS CONTAINING AN MPL LIGAND

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: AMGEN INC.
(B) STREET: 1849 DeHavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: US
(F) ZIP: 91320-1789

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US (Not Yet Assigned)
(B) FILING DATE: 04-OCT-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: COOK Ph.D., Robert R.
(B) REGISTRATION NUMBER: 31,602
(C) REFERENCE/DOCKET NUMBER: A-412

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1342 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 36..1097

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 99..1097

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 36..98

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CAGGGAGCCA CGCCAGCCAA GACACCCCGG CCAGA ATG GAG CTG ACT GAA TTG          53
                                       Met Glu Leu Thr Glu Leu
                                       -21 -20

CTC CTC GTG GTC ATG CTT CTC CTA ACT GCA AGG CTA ACG CTG TCC AGC        101
Leu Leu Val Val Met Leu Leu Leu Thr Ala Arg Leu Thr Leu Ser Ser
-15                 -10                 -5                  1

CCG GCT CCT CCT GCT TGT GAC CTC CGA GTC CTC AGT AAA CTG CTT CGT        149
Pro Ala Pro Pro Ala Cys Asp Leu Arg Val Leu Ser Lys Leu Leu Arg
             5                  10                  15

GAC TCC CAT GTC CTT CAC AGC AGA CTG AGC CAG TGC CCA GAG GTT CAC        197
Asp Ser His Val Leu His Ser Arg Leu Ser Gln Cys Pro Glu Val His
        20                  25                  30

CCT TTG CCT ACA CCT GTC CTG CTG CCT GCT GTG GAC TTT AGC TTG GGA        245
Pro Leu Pro Thr Pro Val Leu Leu Pro Ala Val Asp Phe Ser Leu Gly
    35                  40                  45

GAA TGG AAA ACC CAG ATG GAG GAG ACC AAG GCA CAG GAC ATT CTG GGA        293
Glu Trp Lys Thr Gln Met Glu Glu Thr Lys Ala Gln Asp Ile Leu Gly
50                  55                  60                  65

GCA GTG ACC CTT CTG CTG GAG GGA GTG ATG GCA GCA CGG GGA CAA CTG        341
Ala Val Thr Leu Leu Leu Glu Gly Val Met Ala Ala Arg Gly Gln Leu
                70                  75                  80

GGA CCC ACT TGC CTC TCA TCC CTC CTG GGG CAG CTT TCT GGA CAG GTC        389
Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly Gln Leu Ser Gly Gln Val
            85                  90                  95

CGT CTC CTC CTT GGG GCC CTG CAG AGC CTC CTT GGA ACC CAG CTT CCT        437
Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu Leu Gly Thr Gln Leu Pro
        100                 105                 110

CCA CAG GGC AGG ACC ACA GCT CAC AAG GAT CCC AAT GCC ATC TTC CTG        485
Pro Gln Gly Arg Thr Thr Ala His Lys Asp Pro Asn Ala Ile Phe Leu
    115                 120                 125
```

```
AGC TTC CAA CAC CTG CTC CGA GGA AAG GTG CGT TTC CTG ATG CTT GTA        533
Ser Phe Gln His Leu Leu Arg Gly Lys Val Arg Phe Leu Met Leu Val
130                 135                 140                 145

GGA GGG TCC ACC CTC TGC GTC AGG CGG GCC CCA CCC ACC ACA GCT GTC        581
Gly Gly Ser Thr Leu Cys Val Arg Arg Ala Pro Pro Thr Thr Ala Val
                150                 155                 160

CCC AGC AGA ACC TCT CTA GTC CTC ACA CTG AAC GAG CTC CCA AAC AGG        629
Pro Ser Arg Thr Ser Leu Val Leu Thr Leu Asn Glu Leu Pro Asn Arg
            165                 170                 175

ACT TCT GGA TTG TTG GAG ACA AAC TTC ACT GCC TCA GCC AGA ACT ACT        677
Thr Ser Gly Leu Leu Glu Thr Asn Phe Thr Ala Ser Ala Arg Thr Thr
        180                 185                 190

GGC TCT GGG CTT CTG AAG TGG CAG CAG GGA TTC AGA GCC AAG ATT CCT        725
Gly Ser Gly Leu Leu Lys Trp Gln Gln Gly Phe Arg Ala Lys Ile Pro
    195                 200                 205

GGT CTG CTG AAC CAA ACC TCC AGG TCC CTG GAC CAA ATC CCC GGA TAC        773
Gly Leu Leu Asn Gln Thr Ser Arg Ser Leu Asp Gln Ile Pro Gly Tyr
210                 215                 220                 225

CTG AAC AGG ATA CAC GAA CTC TTG AAT GGA ACT CGT GGA CTC TTT CCT        821
Leu Asn Arg Ile His Glu Leu Leu Asn Gly Thr Arg Gly Leu Phe Pro
                230                 235                 240

GGA CCC TCA CGC AGG ACC CTA GGA GCC CCG GAC ATT TCC TCA GGA ACA        869
Gly Pro Ser Arg Arg Thr Leu Gly Ala Pro Asp Ile Ser Ser Gly Thr
            245                 250                 255

TCA GAC ACA GGC TCC CTG CCA CCC AAC CTC CAG CCT GGA TAT TCT CCT        917
Ser Asp Thr Gly Ser Leu Pro Pro Asn Leu Gln Pro Gly Tyr Ser Pro
        260                 265                 270

TCC CCA ACC CAT CCT CCT ACT GGA CAG TAT ACG CTC TTC CCT CTT CCA        965
Ser Pro Thr His Pro Pro Thr Gly Gln Tyr Thr Leu Phe Pro Leu Pro
    275                 280                 285

CCC ACC TTG CCC ACC CCT GTG GTC CAG CTC CAC CCC CTG CTT CCT GAC       1013
Pro Thr Leu Pro Thr Pro Val Val Gln Leu His Pro Leu Leu Pro Asp
290                 295                 300                 305

CCT TCT GCT CCA ACG CCC ACC CCT ACC AGC CCT CTT CTA AAC ACA TCC       1061
Pro Ser Ala Pro Thr Pro Thr Pro Thr Ser Pro Leu Leu Asn Thr Ser
                310                 315                 320

TAC ACC CAC TCC CAG AAT CTG TCT CAG GAA GGG TAA GGTTCTCAGA            1107
Tyr Thr His Ser Gln Asn Leu Ser Gln Glu Gly  *
            325                 330

CACTGCCGAC ATCAGCATTG TCTCGTGTAC AGCTCCCTTC CCTGCAGGGC GCCCCTGGGA    1167
```

GACAACTGGA CAAGATTTCC TACTTTCTCC TGAAACCCAA AGCCCTGGTA AAAGGGATAC 1227

ACAGGACTGA AAAGGGAATC ATTTTTCACT GTACATTATA AACCTTCAGA AGCTATTTTT 1287

TTAAGCTATC AGCAATACTC ATCAGAGCAG CTAGCTCTTT GGTCTATTTT CTGCA 1342

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Leu Thr Glu Leu Leu Leu Val Val Met Leu Leu Leu Thr Ala
-21 -20                 -15                 -10

Arg Leu Thr Leu Ser Ser Pro Ala Pro Pro Ala Cys Asp Leu Arg Val
 -5                   1                   5                  10

Leu Ser Lys Leu Leu Arg Asp Ser His Val Leu His Ser Arg Leu Ser
             15                  20                  25

Gln Cys Pro Glu Val His Pro Leu Pro Thr Pro Val Leu Leu Pro Ala
         30                  35                  40

Val Asp Phe Ser Leu Gly Glu Trp Lys Thr Gln Met Glu Glu Thr Lys
     45                  50                  55

Ala Gln Asp Ile Leu Gly Ala Val Thr Leu Leu Leu Glu Gly Val Met
 60                  65                  70                  75

Ala Ala Arg Gly Gln Leu Gly Pro Thr Cys Leu Ser Ser Leu Leu Gly
                 80                  85                  90

Gln Leu Ser Gly Gln Val Arg Leu Leu Leu Gly Ala Leu Gln Ser Leu
             95                  100                 105

Leu Gly Thr Gln Leu Pro Pro Gln Gly Arg Thr Thr Ala His Lys Asp
        110                 115                 120

Pro Asn Ala Ile Phe Leu Ser Phe Gln His Leu Leu Arg Gly Lys Val
    125                 130                 135

Arg Phe Leu Met Leu Val Gly Gly Ser Thr Leu Cys Val Arg Arg Ala
140                 145                 150                 155
```

```
Pro Pro Thr Thr Ala Val Pro Ser Arg Thr Ser Leu Val Leu Thr Leu
                160                 165                 170

Asn Glu Leu Pro Asn Arg Thr Ser Gly Leu Leu Glu Thr Asn Phe Thr
            175                 180                 185

Ala Ser Ala Arg Thr Thr Gly Ser Gly Leu Leu Lys Trp Gln Gln Gly
        190                 195                 200

Phe Arg Ala Lys Ile Pro Gly Leu Leu Asn Gln Thr Ser Arg Ser Leu
    205                 210                 215

Asp Gln Ile Pro Gly Tyr Leu Asn Arg Ile His Glu Leu Leu Asn Gly
220                 225                 230                 235

Thr Arg Gly Leu Phe Pro Gly Pro Ser Arg Arg Thr Leu Gly Ala Pro
                240                 245                 250

Asp Ile Ser Ser Gly Thr Ser Asp Thr Gly Ser Leu Pro Pro Asn Leu
            255                 260                 265

Gln Pro Gly Tyr Ser Pro Ser Pro Thr His Pro Pro Thr Gly Gln Tyr
        270                 275                 280

Thr Leu Phe Pro Leu Pro Pro Thr Leu Pro Thr Pro Val Val Gln Leu
    285                 290                 295

His Pro Leu Leu Pro Asp Pro Ser Ala Pro Thr Pro Thr Pro Thr Ser
300                 305                 310                 315

Pro Leu Leu Asn Thr Ser Tyr Thr His Ser Gln Asn Leu Ser Gln Glu
                320                 325                 330

Gly  *
```

—

WHAT IS CLAIMED IS:

1. A composition comprising an mpl ligand, a buffer selected from the group consisting of glutamate, phosphate, histidine, imidazole, and acetate; an excipient selected form the group consisting of sorbitol, sucrose, mannitol, glycerol, polyethylene glycol, and a non-polar amino acid; and having a pH ranging from 5.0 to 6.0, inclusive.

2. A composition according to Claim 1, wherein the mpl ligand comprises at least amino acids 7-151 of SEQ ID. NO: 2.

3. A composition according to Claim 1, wherein the mpl ligand consists of amino acids 1-171 ± 20 amino acids of SEQ ID NO: 2.

4. A composition according to Claim 1, wherein the mpl ligand consists of amino acids 1-161 ± 10 amino acids of SEQ ID NO: 2.

5. A composition according to Claim 1, wherein the mpl ligand consists of amino acids 1-151 amino acids of SEQ ID NO: 2.

6. A composition according to Claim 1, wherein the mpl ligand consists of amino acids 1-163 of SEQ ID NO: 2.

7. A composition according to Claim 1, wherein the mpl ligand is attached to a water soluble polymer selected from the group consisting of polyethylene glycol, monomethoxy-polyethylene glycol, dextran, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol

homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols.

8. A composition according to Claim 7, wherein the water soluble polymer is polyethylene glycol.

9. A composition according to Claim 6, which comprises acetate as the buffer, sorbitol as the excipient, and has a pH of about 5.0, in an aqueous medium.

10. A composition according to Claim 1, wherein the nonpolar amino acid is selected form the group consisting of glycine, proline, and alanine

11. A composition according to Claim 1, further comprising an antioxidant.

12. A composition according to Claim 11, wherein the antioxidant is selected from the group consisting of EDTA, ascorbic acid, glutathione, methionine, and citrate.

13. A composition according to Claim 1, further comprising a detergent or a lipid.

14. A composition according to Claim 13, wherein the detergent is selected from the group consisting of Tween; Brij 35; Pluronics; sodium dodecyl sulfate; Triton; dimyristoyl phosphatidyl glycerol (DMPG); PEG-40 castor oil; oleth-3-phosphate; diethanolamine oleth-10-phosphate; and a mixture of short and long chain unilamellar vesicles (SLUV) containing C8 (caprylic) and C14 (myristic) lipids.

15. A composition according to Claim 1, wherein the composition is in an aqueous medium.

16. A composition according to Claim 1, wherein the composition is in a lyophilized form.

17. A composition according to Claim 1, which comprises phosphate buffer, 5% sorbitol and has a pH of about 6.0, in an aqueous medium.

18. A composition according to Claim 1, which comprises histidine buffer, and about 5% sorbitol in an aqueous medium.

19. A composition according to Claim 1, which comprises imidazole buffer, and about 5% sorbitol in an aqueous medium.

20. A composition according to Claim 1, which comprises glutamate buffer, and about 5% sorbitol, in an aqueous medium.

21. A composition according to Claim 1, which comprises glutamate buffer, 5% sorbitol and has a pH of about 5.0, in an aqueous medium.

22. A composition according to Claim 1, which comprises glutamate buffer, about 6% sucrose, about 2% mannitol, a pH of about 5.0 in a lyophilized form.

```
  1     CAGGGAGCCACGCCAGCCAAGACACCCCGGCCAGAATGGAGCTGACTGAATTGCTCCTC   59
-21                                          MetGluLeuThrGluLeuLeuLeu  -14

 60   GTGGTCATGCTTCTCCTAACTGCAAGGCTAACGCTGTCCAGCCCGGCTCCTCCTGCTTGT   119
-13   ValValMetLeuLeuLeuThrAlaArgLeuThrLeuSerSerProAlaProProAlaCys    7

120   GACCTCCGAGTCCTCAGTAAACTGCTTCGTGACTCCCATGTCCTTCACAGCAGACTGAGC   179
 8   AspLeuArgValLeuSerLysLeuLeuArgAspSerHisValLeuHisSerArgLeuSer    27

180   CAGTGCCCAGAGGTTCACCCTTTGCCTACACCTGTCCTGCTGCCTGCTGTGGACTTTAGC   239
 28   GlnCysProGluValHisProLeuProThrProValLeuLeuProAlaValAspPheSer    47

240   TTGGGAGAATGGAAAACCCAGATGGAGGAGACCAAGGCACAGGACATTCTGGGAGCAGTG   299
 48   LeuGlyGluTrpLysThrGlnMetGluGluThrLysAlaGlnAspIleLeuGlyAlaVal    67

300   ACCCTTCTGCTGGAGGGAGTGATGGCAGCACGGGGACAACTGGGACCCACTTGCCTCTCA   359
 68   ThrLeuLeuLeuGluGlyValMetAlaAlaArgGlyGlnLeuGlyProThrCysLeuSer    87

360   TCCCTCCTGGGGCAGCTTTCTGGACAGGTCCGTCTCCTCCTTGGGGCCCTGCAGAGCCTC   419
 88   SerLeuLeuGlyGlnLeuSerGlyGlnValArgLeuLeuLeuGlyAlaLeuGlnSerLeu   107
```

FIG. 1A

```
420   CTTGGAACCCAGCTTCCTCACAGGGCAGGACCACAGCTCACAAGGATCCCAATGCCATCC   479
108   LeuGlyThrGlnLeuProProGlnGlyArgThrThrAlaHisLysAspProAsnAlaIle   127

480   TTCCTGAGCTTCCAACACCTGCTCCGAGGAAAGGTGCGTTTCCTGATGCTTGTAGGAGGG   539
128   PheLeuSerPheGlnHisLeuLeuArgGlyLysValArgPheLeuMetLeuValGlyGly   147

540   TCCACCCTCTGCGTCAGGCGGGCCCCACCCACCACAGCTGTCCCCAGCAGAACCTCTCTA   599
148   SerThrLeuCysValArgArgAlaProProThrThrAlaValProSerArgThrSerLeu   167

600   GTCCTCACACTGAACGAGCTCCCAAACAGGACTTCTGGATTGTTGGAGACAAACTTCACT   659
168   ValLeuThrLeuAsnGluLeuProAsnArgThrSerGlyLeuLeuGluThrAsnPheThr   187

660   GCCTCAGCCAGAACTACTGGCTCTGGGCTTCTGAAGTGGCAGCAGGGATTCAGAGCCAAG   719
188   AlaSerAlaArgThrThrGlySerGlyLeuLeuLysTrpGlnGlnGlyPheArgAlaLys   207

720   ATTCCTGGTCTGCTGAACCAAACCTCCAGGTCCCTGGACCAAATCCCCGGATACCTGAAC   779
208   IleProGlyLeuLeuAsnGlnThrSerArgSerLeuAspGlnIleProGlyTyrLeuAsn   227

780   AGGATACACGAACTCTTGAATGGAACTCGTGGACTCTTTCCTGGACCCTCACGCAGGACC   839
228   ArgIleHisGluLeuLeuAsnGlyThrArgGlyLeuPheProGlyProSerArgArgThr   247
```

FIG.1B

```
 840    CTAGGAGCCCCGGACATTTCCTCAGGAACATCAGACACAGGCTCCCTGCCACCCAACCTC    899
 248    LeuGlyAlaProAspIleSerSerGlyThrSerAspThrGlySerLeuProProAsnLeu    267

 900    CAGCCTGGATATTCTCCTTCCCCAACCCATCCTCCTACTGGACAGTATACGCTCTTCCCT    959
 268    GlnProGlyTyrSerProSerProThrHisProProThrGlyGlnTyrThrLeuPhePro    287

 960    CTTCCACCCACCTTGCCCACCCCTGTGGTCCAGCTCCACCCCCTGCTTCCTGACCCTTCT   1019
 288    LeuProProThrLeuProThrProValValGlnLeuHisProLeuLeuProAspProSer    307

1020    GCTCCAACGCCCACCCCTACCAGCCCTCTTCTAAACACATCCTACACCCACTCCCAGAAT   1079
 308    AlaProThrProThrProThrSerProLeuLeuAsnThrSerTyrThrHisSerGlnAsn    327

1080    CTGTCTCAGGAAGGGTAAGGTTCTCAGACACTGCCGACATCAGCATTGTCTCGTGTACAG   1139
 328    LeuSerGlnGluGlyEnd                                              332
1140    CTCCCTTCCCTGCAGGGCGCCCCTGGGAGACAACTGGACAAGATTTCCTACTTTCTCCTG   1199
1200    AAACCCAAAGCCCTGGTAAAAGGGATACACAGGACTGAAAAGGGAATCATTTTTCACTGT   1259
1260    ACATTATAAACCTTCAGAAGCTATTTTTTTAAGCTATCAGCAATACTCATCAGAGCAGCT   1319
1320    AGCTCTTTGGTCTATTTTCTGCA    1342
```

FIG.1C

INTERNATIONAL SEARCH REPORT

Inter. ...nal Application No
PCT/US 97/16196

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/52 A61K38/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 97 26907 A (GENENTECH INC ;THOMAS GRIFFITH R (US)) 31 July 1997 whole document, esp. page 9ff, page 40ff, experiment 3 ---	1
P,X P,Y	WO 96 40217 A (ZYMOGENETICS INC) 19 December 1996 whole document, especially claim 25 and examples --- -/--	1,7,15 2-6, 8-14, 16-22

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
8 January 1998	23. 01. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kronester-Frei, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna .al Application No
PCT/US 97/16196

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 96 41642 A (KIRIN BREWERY ;NOMURA HIDEAKI (JP)) 27 December 1996	1,6
P,Y	whole document, esp. page 33ff	2-5,7-22
P,X	& CHEMICAL ABSTRACTS, vol. 126, no. 9, 3 March 1997 Columbus, Ohio, US; abstract no. 122491,	1,6
P,Y	see abstract	2-5,7-22

X	WO 96 29989 A (AMGEN INC) 3 October 1996	1-22
Y	whole document, esp. page 18ff, claims 21-24, examples	1-22

Y	WO 95 26746 A (AMGEN INC ;BARTLEY TIMOTHY D (US); BOGENBERGER JAKOB M (US); BOSSE) 12 October 1995 cited in the application whole document, esp. page 53ff, Table 7, claims	1-22

P,Y	KUTER D.J. : "In vivo effects of Mpl ligand administration and emerging clinical applications for the Mpl ligands" CURRENT OPINION IN HEMATOLOGY, vol. 4, no. 3, May 1997, pages 163-170, XP002051381 whole document	1-22

A	WO 96 25498 A (AMGEN INC ;ELLIOTT STEVEN G (US)) 22 August 1996 whole document, esp. page 17, line 21ff, page 20, lines 10-17, claim 21	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna. .al Application No
PCT/US 97/16196

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9726907 A	31-07-97	AU 1533597 A	20-08-97
WO 9640217 A	19-12-96	AU 5802396 A	30-12-96
WO 9641642 A	27-12-96	AU 5911796 A	09-01-97
WO 9629989 A	03-10-96	AU 5434196 A	16-10-96
		NO 974323 A	28-11-97
WO 9526746 A	12-10-95	AU 2230895 A	23-10-95
		BG 100625 A	30-04-97
		CN 1137757 A	11-12-96
		CZ 9503505 A	15-01-97
		EP 0675201 A	04-10-95
		EP 0690127 A	03-01-96
		EP 0755263 A	29-01-97
		FI 960136 A	11-03-96
		HU 74257 A	28-11-96
		NO 960111 A	24-09-96
		PL 312577 A	29-04-96
		SK 166695 A	05-02-97
		ZA 9502651 A	25-06-96
		ZA 9502652 A	21-12-95
WO 9625498 A	22-08-96	US 5696250 A	09-12-97
		AU 4994696 A	04-09-96
		CA 2209298 A	22-08-96
		EP 0811064 A	10-12-97